1. LITERATURE REVIEW

1.1- Background

Ayurveda, the ancient healing system of India, flourished in the Vedic era in India. According to historical facts, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were written around 1000B.C. The Ayurvedic Materia Medica includes 600 medicinal plants along with therapeutics. Herbs like turmeric, fenugreek, ginger, garlic and holy basil are integral part of Ayurvedic formulations.

Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. Natural products are more reliable than their synthetic counterparts due to their minimal side effects.

From the 1920's to 30's herbal medicine went into decline as discoveries of various chemical drugs and antibiotics were discovered. From the late 1920's till 1960's, the use of herbs was rapidly dropped from the United States Pharmacopoeia. Their reemergence reoccurred in the late 1960's, as increasing numbers of people were experiencing side effects from conventional drugs. Today we find pharmaceutical companies rapidly entering the herbal market. The potential market is huge and growing incrementally.

In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. Isolated active constituents are used for applied research. For the last few decades, phytochemistry (study of plants) has been making rapid progress and herbal products are becoming popular. The prevalence of population suffering from stress related disorders clubbed with hypertension is quite alarming. With this view in mind, I proposed to study the anxiolytic and antihypertensive effects of some Indian medicinal plants and make an attempt to elucidate its possible mode of action.

1.2 - Plants selected for the study include

1.2.1- *Trigonella foenum- graecum* Linn.

Family: Leguminosae

Part used: seeds

Synonyms: Fenugreek, Bockshornsame

Constituents:

Alkaloids (pyridine type)- Gentianine, trigonelline (upto 0.13%), choline (0.05%)

Proteins and amino acids- Protein (23-25%) containing high quantities of lysine and tryptophan. Free amino acids include 4-hydroxyisoleucine (0.09%), histidine, lysine and arginine.

Flavanoids- Flavone (apigenin,luteolin) glycosides including orientin and vitexin, quercetin (flavonol)

Saponins (0.6-1.7%)- Glycosides yielding steroidal sapogenins diosgenin and yamogenin (major) with tigogenin and neotigogenin, gitogenin, neogitogenin, smilagenin, sarsasapogenin, yuccagenin; fenugreekine a sapogenin peptide ester involving diosgenin and yamogenin; trigofenosides.

Other constituents- Coumarin, lipids (5-8%), mucilaginous fibre (50%), vitamins and minerals.

Medicinal actions:

Trigonella foenum-graecum L. (Leguminosae) commonly known as Fenugreek is native to the area from the eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in Pakistan, India and China (Morton, 1990). The seeds have hot and sharp bitter taste. They are used for their carminative, tonic, laxative and expectorant properties. They possess antipyretic, anthelmentic, appetite stimulant (Kirtikar and Basu, 1993a), aphrodisiac (Chopra *et al.*, 1982), antifatigue (Jha, 2003), hypocholesterolaemic and hypoglycaemic activity (Sharma, 1986; Ribes, 1984). Fenugreek seeds and leaves are also said to have antidiabetic (Shani *et al.*, 1974), antiulcer (Al Meshal *et al.*, 1985) and antihypertensive properties (Ghosal *et al.*, 1974). The seeds of *Trigonella foenum-graecum* contain the alkaloid

trigonelline with mucilage, tannic acid, yellow colouring matter, fixed and volatile oils and a bitter extractive diosgenin, gitogenin a trace of trigogenin and Vitamin A (Jayaweera, 1981).

1.2.2 - *Zingiber officinale* Rosc.

Family: Zingiberaceae
Part used: rhizome
Synonym: Ginger
Constituents:
Carbohydrates- starch (major constituent upto 50%)
Lipids (6-8%)- free fatty acids, triglycerides, phosphatidic acid, lecithins
Oleoresin- Gingerol homologues (major about 33%), shogaol homologues (dehydration products of gingerols), zingerone (degradation product of gingerols), volatile oils
Volatile oils (1-3%)- Predominantly hydrocarbons. β–Bisabolene and Zingiberene (major); other sesquiterpenes include zingiberol, zingiberenol, ar-curcumene, β-sesquiphellandrene, β-sesquiphellandrol; numerous monoterpene hydrocarbons, alcohols and aldehydes
Other constituents- Amino acids, proteins (about 9%), resins, vitamins and minerals
Medicinal actions:
The rhizome part of *Zingiber officinale* (Zingiberaceae) commonly known as ginger possesses carminative, laxative, expectorant (Kirtikar and Basu, 1993b), hypocholesterolaemic (Giri *et al.*, 1984), and hypoglycaemic activity (Sharma and Shukla, 1977). The 5-HT_3 receptor antagonistic properties (Yamahara *et al.*, 1989) of ginger accounts for its anti-emetic effects (Bone *et al.*, 1990). It is effective in emesis induced in cancer chemotherapy (Sharma and Gupta, 1988), motion sickness (Qian and Liu, 1992), sea sickness (Gronived *et al.*, 1998) and pregnancy (Fischer *et al.*, 1991). Ginger has been reported to have hypo- and hypertensive, cardiac (Suekawa *et al.*, 1986), blood pressure lowering effects (Ghayur *et al.*, 2005a; 2005b) and prostaglandin (Kiuchi *et al.*, 1982), platelet aggregation inhibition (Srivastava; 1984a, 1984b), cholagogic (Yamahara *et al.*, 1985), stomachic properties (Yamahara *et al.*, 1988) and anxiolytic and anti-emetic activity (Vishwakarma *et al.*, 2002).

1.2.3- *Panax pseudoginseng* Wallich. and *Korean Ginseng* Linn.

Family: Araliaceae

Parts used: roots/ rhizome

Synonyms: Panax ginseng, Chinese ginseng, Asiatic ginseng, Japanese ginseng and Oriental ginseng

Common name: Ginseng

Constituents:

Terpenoids- It contains triterpene glycosides named ginsenosides which account for the majority of plants medicinal action. At least 13 ginsenosides have been identified falling into 2 groups based upon the aglycone portion: protopanaxodiol (diols) and protopanaxatriol (triols). They are classified according to an alphanumeric system i.e. Ra, Rb, Rb2, Rc, etc.

Other constituents- The plant also contains volatile oil (trace), sterols, acetylenic compounds and peptidoglycans named Panaxans (Mills, 1991; Wren, 1988).

Panax pseudoginseng is a rich source of oleanolic acid saponins while dammarane saponins are present in minor amounts (Shukla and Thakur, 1986; Shukla and Thakur, 1988; Kim *et al.,* 1995). The active constituents of Korean ginseng like ginsenosides, Rb2, Re, Rgi and chikusetsusaponin IV, V are also present in *Indian pseudoginseng* (Shukla and Thakur, 1986).

Medicinal actions:

The roots and the flowers of *Panax pseudoginseng* are antibacterial, anti-inflammatory, antiseptic, cardiotonic, diuretic, haemostatic, and hypoglycaemic (Yeung, 1985; Bown, 1995). The root is used internally in the treatment of coronary heart disease and angina (Bown, 1995). It also has adaptogenic (Dua *et al.*, 1989), anti-ulcer (Sun *et al.*, 1992), antihepatotoxic (Hikino *et al.*, 1985), antinarcotic (Kim *et al.*, 1990) and antiplatelet actions (Teng *et al.*, 1989). Ginseng is also being used as one of the commonly used over the counter herbal prescription in patients with cardiovascular disease. (Pharand *et al.*, 2003).

Korean (Panax) ginseng (Araliaceae) has a blood pressure lowering effect (Stavro *et al.*, 2004; Jeon *et al.*, 2000; Han *et al.*, 1998), antistress (Bhattacharya and Sur, 1999) and anabolic activity (Grandhi *et al.*, 1994) and improves learning and memory (Sung-Ha Jui *et al.*, 1999). It has a true adaptogenic action. *Korean ginseng* affects the immune system,

increasing the function of reticuloendothelial system, stimulating phagocytic function and preventing some viral infections. (Bensky *et al.*, 1986, Hsou-Mou Chang, 1986) Ginseng administration in animals has shown behavioral changes, which seem to be related to the regulation of gamma-amino-butyric acid (GABA) ergic neurotransmission. Ginseng saponin prolonged pentobarbitone sleeping time and delayed the onset of convulsion in high dose (Jung and Jin, 1996; Oh *et al.*, 1969). Ginsenoside Re is a potent inhibitor of neurotransmitter inhibitor, specially, GABA (Tsang *et al.*, 1983). Ginsenosides interact with ligand- bindings of $GABA_A$ and $GABA_B$ receptors (Kimura *et al.*, 1994). Ginseng also has anxiolytic effects (Hwa-Young Cha *et al.*, 2004).

In the present study, saponins of the Indian variety of ginseng- *Panax pseudoginseng* has been studied for anxiolytic and antihypertensive effects and compared with saponins of its Korean counterpart- *Korean ginseng*. Anxiolytic effects of both the plant extracts have been compared against diazepam.

In search for better alternatives to antihypertensive and anxiolytic drugs, the 5-HT class of drugs namely; the $5\text{-}HT_3$ receptor antagonists, $5\text{-}HT_{1A}$ agonist and $5\text{-}HT_{2B}$ antagonists are currently being considered for their potential use in hypertension (Tsukomoto *et al.*, 2000; Shingala and Balaraman, 2005). Reports on anxiolytic studies on the above selected plants are very few (Vishwakarma *et al.*, 2002; Hwa-Young Cha *et al.*, 2004). I therefore proposed to study anxiety and antihypertensive properties of the above plant species through its possible 5-HT mechanism.

1.3- 5-HT receptors

At the beginning of last century, Brodie (1900) described in an extensive study that injection of blood serum causes vasoconstriction and a vagally- mediated reflex resulting in a reversible bradycardia, hypotension and arrest of the respiration, while injection of blood plasma was devoid of these effects. About 50 years ago, the hormone and neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) was isolated from blood serum and named due to its origin and vascular action (sero= serum and tonin= vasoconstriction) (Rapport *et al.*, 1948). Similarly, another endogenous substance from the enterochromaffin cells (present in gastrointestinal mucosa) was isolated, functionally characterised and named enteramine (Erspamer and Asero, 1952). The functional properties of enteramine were mainly smooth muscle contraction and further investigations revealed that enteramine and serotonin were chemically and pharmacologically similar (Erspamer, 1954).

1.3.1 - Classification and nomenclature of 5-HT receptors

At the end of 1950's, it was reported that serotonin produces smooth muscle contraction in guinea pig ileum that was mediated by two different receptors, one sensitive to morphine (5-HT-M) and another to dibenzyline (phenoxybenzamine; 5-HT-D); this was the first evidence for the existence of multiple receptors for serotonin (Gaddum and Picarelli, 1957). For nearly three decades, the further characterisation of these receptor types was hampered, although some report stated that non-5-HT-M/D receptors were involved in canine carotid vasoconstriction (Saxena, 1972). The discovery of radioligand binding techniques provided important information for further characterisation of 5-HT receptors. Thus, the presence of two different receptor subtypes (5-HT1 and 5-HT2), preferentially labelled by [^{3}H] 5-HT and [^{3}H] spiperone, respectively, were reported in the brain homogenate (Peroutka and Snyder, 1979).

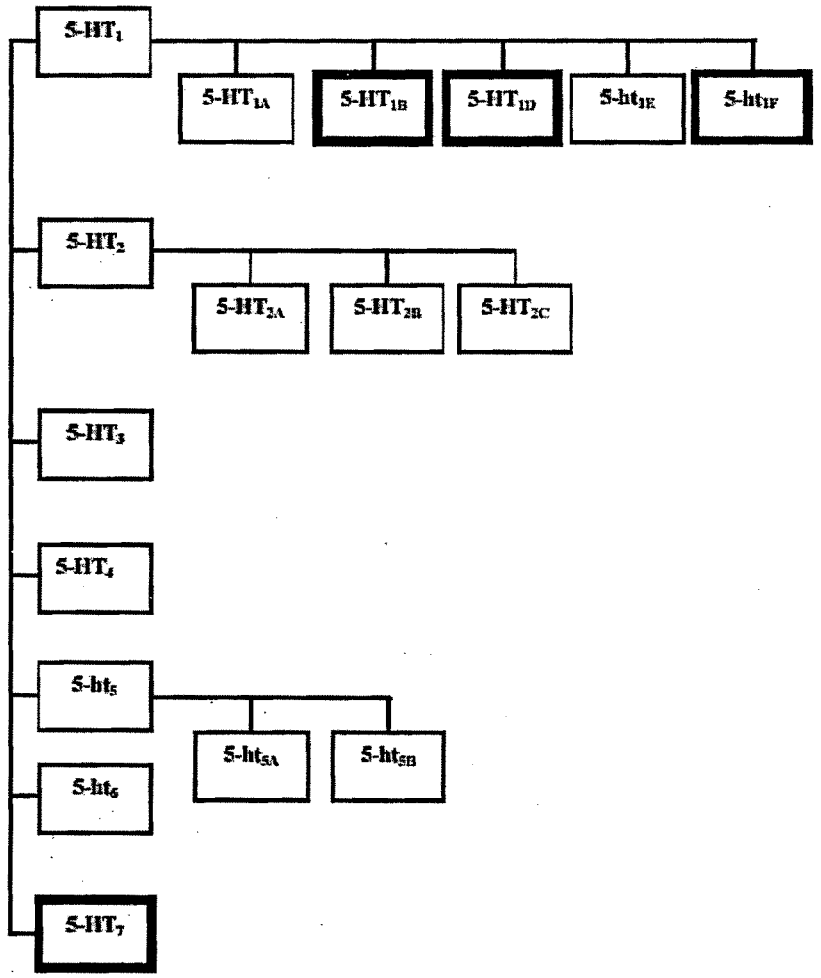


Figure 1: NC-IUPHAR (Serotonin receptor nomenclature committee of the International union of Pharmacology) serotonin (5-HT) receptor classification. Lower case letters represent recombinant receptors without well-known functional characterisation, whereas upper case letters denote well-characterised receptors (Hoyer *et al.*, 1994).

In 1986, Bradley *et al.* proposed a different classification and nomenclature for 5-HT receptors, where three different 5-HT receptor subtypes were identified, namely: **1)** '5-HT_1-like' receptors, which displayed high affinity for 5-carboxamidotryptamine and could be labelled with [^{3}H] 5-HT; **2)** 5-HT_2 receptors, being identical to the 5-HT-D receptor and displayed high affinity for ketanserin; and **3)** 5-HT_3 receptors, which are ion channels and

identical to the 5-HT-M receptor (Bradley *et al.*, 1986). However, with the introduction of several molecular biological tools and their application together with biochemical and functional techniques in the last decade led to an integrated approach for characterising receptors on the basis of amino acid composition, linkage to second messengers as well as functional responses. Several novel 5-HT receptors were identified, characterised and even sometimes named differently in different laboratories (e.g. 5-HT_{1B} receptors were also known as $5\text{-HT}_{1D\beta}$ or S12) (Levy *et al.*, 1992; Weinshank *et al.*, 1992). To overcome this ambiguity, the Serotonin Receptor Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR) reclassified 5-HT receptors into seven subclasses, based on the modern criteria (Figure 1) (Hoyer *et al.*, 1994).

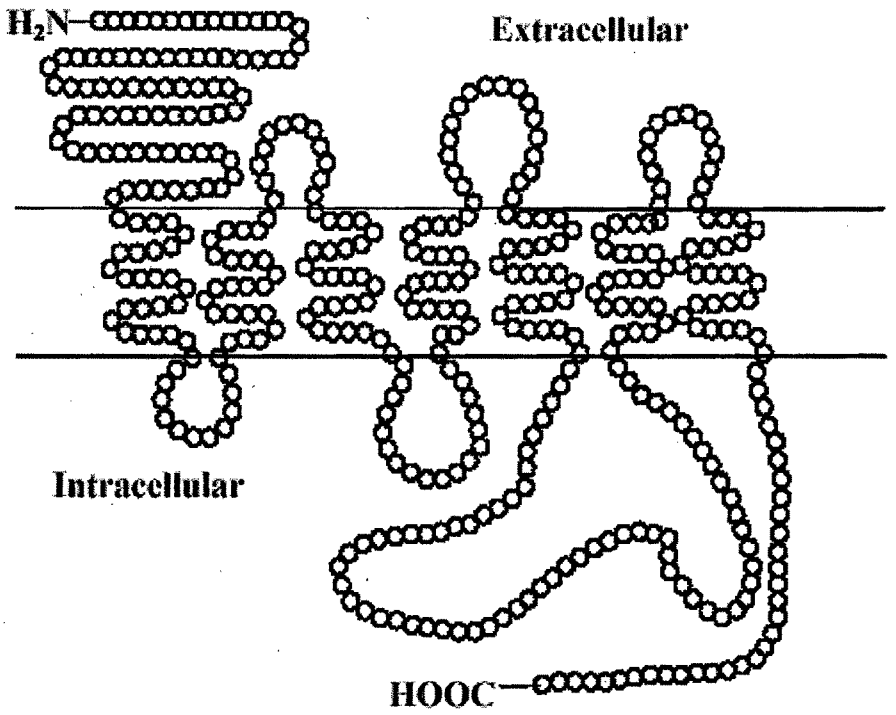


Figure 2: Schematic representation of a GPCR (G- Protein coupled receptor). The circles represent aminoacids and both N- and C-terminals are given as NH2- and COOH-group, respectively.

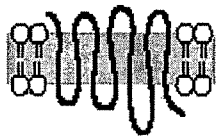
Basically on the basis of their molecular structure, all 5-HT receptors (except 5-HT_3 receptor) are members of the G-protein-coupled receptor superfamily (GPCRs), which consist of integral membrane proteins and interact with a large variety of hormones and neurotransmitters (Iismaa *et al.*, 1995). A common feature of GPCRs is the seven transmembrane domains spanning the cell membrane, having the N-terminal on the extracellular side, while the C-terminal is present on the intracellular side. Mainly the C-terminal cytoplasmatic tail appears to be important as phoshorylation site where specific kinase enzyme catalyse the coupling of phosphate groups (Iismaa *et al.*, 1995). Furthermore, it has become clear that the active binding site for ligands (agonists and antagonists) in these proteins are mainly located in the transmembrane regions (called α-helices) of the protein, as observed by site directed mutagenesis and chimeric receptors, e.g. in 5-HT_1 receptors (Adham *et al.*, 1994; Oksenberg *et al.*, 1992; Wurch *et al.*, 1998). After receptor activation and G-protein coupling, a number of possible signal transduction pathways have been described for GPCRs (Iismaa *et al.*, 1995). The 5-HT receptors couple to the enzymes adenylyl cyclase (which promotes the production of cyclic adenosine monophosphate) and phospholipase C (which promotes the production of inositol triphosphate and increases intracellular calcium) or ion channels (e.g. potassium channels). Since these effector systems are present in every cell, GPCRs play a major important role in the regulation of physiological responses and actions of around 80% of all neurotransmitters and hormones (Birnbaumer *et al.*, 1990).

Table 1

Serotonin Receptor Subtypes

Structural Families

5-HT$_1$, 5-HT$_2$, 5-HT$_{4-7}$



G protein–coupled receptor

5-HT$_3$

5-HT–gated ion channel

SUBTYPE	GENE STRUCTURE	SIGNAL TRANSDUCTION	LOCALIZATION	FUNCTION	SELECTIVE AGONIST	SELECTIVE ANTAGONIST
5-HT$_{1A}$	Intronless	Inhibition of AC	Raphe nuclei Hippocampus	Autoreceptor	8-OH-DPAT	WAY 100135
5-HT$_{1B}$*	Intronless	Inhibition of AC	Subiculum Substantia nigra	Autoreceptor	—	—
5-HT$_{1D}$	Intronless	Inhibition of AC	Cranial blood vessels	Vasoconstriction	Sumatriptan	—
5-HT$_{1E}$	Intronless	Inhibition of AC	Cortex Striatum	—	—	—
5-HT$_{1F}$†	Intronless	Inhibition of AC	Brain and periphery	—	—	—
5-HT$_{2A}$ (D Receptor)	Introns	Activation of PLC	Platelets Smooth muscle Cerebral cortex	Platelet aggregation Contraction Neuronal excitation	α-Methyl-5-HT, DOI	Ketanserin LY53857 MDL 100,907
5-HT$_{2B}$	Introns	Activation of PLC	Stomach fundus	Contraction	α-Methyl-5-HT, DOI	LY53857
5-HT$_{2C}$	Introns	Activation of PLC	Choroid plexus	—	α-Methyl-5-HT, DOI	LY53857 Mesulergine
5-HT$_3$ (M Receptor)	Introns	Ligand-operated ion channel	Peripheral nerves Area postrema	Neuronal excitation	2-Methyl-5-HT	Ondansetron Tropisetron
5-HT$_4$	Introns	Activation of AC	Hippocampus Gastrointestinal tract	Neuronal excitation	Renzapride	GR 113808
5-HT$_{5A}$	Introns	Unknown	Hippocampus	Unknown	—	—
5-HT$_{5B}$	Introns	Unknown			—	—
5-HT$_6$	Introns	Activation of AC	Striatum	Unknown	—	—
5-HT$_7$	Introns	Activation of AC	Hypothalamus Intestine	Unknown	—	—

*Also referred to as 5-HT$_{1Dβ}$.
†Also referred to as 5-HT$_{1Dα}$.
NOTE: AC, adenylyl cyclase; PLC, phospholipase C; 8-OH-DPAT, 8-hydroxy-(2-N,N-dipropylamino)-tetraline; DOI, 1-(2,5-dimethoxy-4-iodophenyl)isopropylamine.

(Taken from Goodman and Gilman "The Pharmacological Basis of Therapeutics" 10th edition, pg. 271)

Table 2

Summary of changes in 5-HT receptor nomenclature

Old nomenclature		**New nomenclature**
Receptor	Species	
5-HT_{1B} 5-HT_{1D} $5\text{-HT}_{1D\beta}$	Rat Human,Guinea Pig All species	5-HT_{1Ba}
$5\text{-HT}_{1D\alpha}$	All species	5-HT_{1D}
5-HT_{2} 5-HT_{D}	All species	5-HT_{2A}
$5\text{-HT}_{2\alpha}$	All species	5-HT_{2B}
5-HT_{1C}	All species	5-HT_{2C}

[Taken from Barnes and Sharp (1999) Neuropharmacology 38, 1083]

1.3.2 - Physiological responses produced by serotonin

5-HT is synthesised both in the intestines and brain from the essential amino acid L-tryptophan. Interestingly, since 5-HT cannot cross the blood-brain barrier, there is a clear distinction between its central and its peripheral functions. Furthermore, 5-HT and its receptors are found both in the central and peripheral nervous system, as well as in a number of non-neuronal tissues in the gut, cardiovascular system and blood. In the central nervous system, 5-HT plays an important role as neurotransmitter (Saxena, 1995) and is involved in appetite, sleep, memory, thermoregulation, sexual behaviour, hallucinations, anxiety and depression. In the periphery, 5-HT plays a role in the aggregations of thrombocytes, smooth muscle contraction, presynaptic transmitter release (neurotransmitter and neuropeptide) and

stimulation of nerve fibres (Martin, 1994). It may be important to note that certain 5-HT_1 receptor subtypes mediate (among others) cranial vasoconstriction and inhibition of neuropeptides release (e.g. substance P, CGRP, neurokinin A), which may be important for their role in migraine therapy (De Vries *et al.*, 1999). In the last decades, it has become clear that the cardiovascular effects of 5-HT are complex and they consist of bradycardia or tachycardia, hypotension or hypertension, vasodilatation and vasoconstriction (Saxena & Villalon, 1990; 1991). In most species, 5-HT-induced bradycardia involves 5-HT_3 receptors, via the so-called von Bezold-Jarisch reflex (Paintal, 1973). On the other hand, tachycardia produced by 5-HT is species-dependent, including a direct or indirect action at 5-HT_2 (rat, dog); 5-HT_3 (rabbit, dog); 5-HT_4 (pig, human); and 5-HT_7 (cat) receptors or by tyramine-like (guinea pig) and unidentified mechanisms (Saxena & Villalon, 1990; Villalón *et al.*, 1997). With respect to the blood pressure changes, intravenous administration of 5-HT causes a triphasic response in anaesthetised animals (with intact vagal nerve), which comprises of: (i) an initial 5-HT_{1A} receptor-mediated hypotension, also due to the bradycardia; (ii) a 5-HT_{2A} receptor-mediated hypertension; and (iii) a long-lasting 5-HT_7 receptor-mediated hypotension (De Vries *et al.*, 1997; De Vries *et al.*, 1999; Kalkman *et al.*, 1984; Saxena & Villalon, 1990). To date, it is known that 5-HT can act at a wide range of receptor, 5-$HT_{1A/B/D/F}$, 5-$HT_{2A/B}$, 5-HT_3, 5-HT_4, 5-$ht_{5A/B}$, 5-ht_6 and 5-HT_7 receptors (Figure 1), by which it can influence different physiological responses. 5-HT has been implicated in the aetiology of numerous disease states, including depression anxiety and social phobia, schizophrenia, obsessive compulsive and panic disorders; in addition to migraine, hypertension, pulmonary hypertension, eating disorders, vomiting and irritable bowel syndrome (Hoyer *et al.*, 1994; Saxena, 1995; Saxena & Villalon, 1990). With the development of more selective ligands at these receptors (agonists and antagonists) and with the application of several molecular biological and pharmacological assays, it may hopefully be possible to elucidate the importance of 5-HT receptor subtypes as potential therapeutic target in the near future.

1.3.3 - Neurobiology of Brain Aversive Systems

A number of different views have been taken as to the organisation of the brain's aversive systems. The following account is an attempt to synthesise these views, in order to understand better how the various behavioural paradigms that have been used to investigate the role of 5-HT fit into the neurobiological picture. The ability to recognise and respond to danger or its warning signals is essential for survival in humans, as well as animals. The brain systems that deal with this permeate all levels of the neuraxis (Figure. 3). Basically, an organism can come to harm in two ways: harm can be externally imposed (such as predation or threat of predation) or it can be the consequence of the actions of the organism itself. Correspondingly, ideas have developed concerning two aversive systems, the (antipredator) Defence System and the Behavioural Inhibition System. The defence system is envisaged as dealing with externally imposed threats such as predation (Fanselow, 1991). At the highest level, the amygdala obtains its inputs from the cortex or the thalamus according to the complexity of the stimulus (Romanski and Ledoux, 1992; Rosen *et al.*, 1992). It is here that conditioned (CS +) and unconditioned aversive stimuli become associated (Romanski *et al.*, 1993). The amygdala can exert control over lower levels, such as the hypothalamic 'defence area' from which integrated and directed flight or aggression can be elicited by electrical stimuli (Kruk, 1991) and which also gives the amygdala access to appropriate cardiovascular and endocrine reactions (LeDoux *et al.*, 1988). The amygdala also has prominent inputs to the periaqueductal grey (PAG). This is one of the most important defensive regions of the brain (Graeff, 1988; Fanselow, 1991). It is functionally and structurally complex but can be divided broadly into two regions-the ventrolateral (VPAG) and dorsal (DPAG) PAG. As well as receiving prominent inputs from higher centres--cortex, limbic system and extrapyramidal motor systems-it has direct access to visual information via the superior colliculus, and this information is available to link context and imminence of danger with choice of defensive response (Redgrave and Dean, 1991). Skin sensors, baroreceptors and chemoreceptors also supply the PAG with information crucial to defence (Fanselow, 1991). DPAG is important in active defensive behaviour explosive motor behaviour, such as 'blind' running and jumping, flight and defensive aggression, together with cardiovascular and respiratory stimulation and naloxone-insensitive analgesia (non-opioid analgesia, NOA). Similarities between DPAG

stimulation and human panic are remarkable (Deakin and Graeff, 1991), and stimulation of this area is extremely aversive in humans, producing intense fear and convictions of impending death (Nashold *et al.*, 1969).

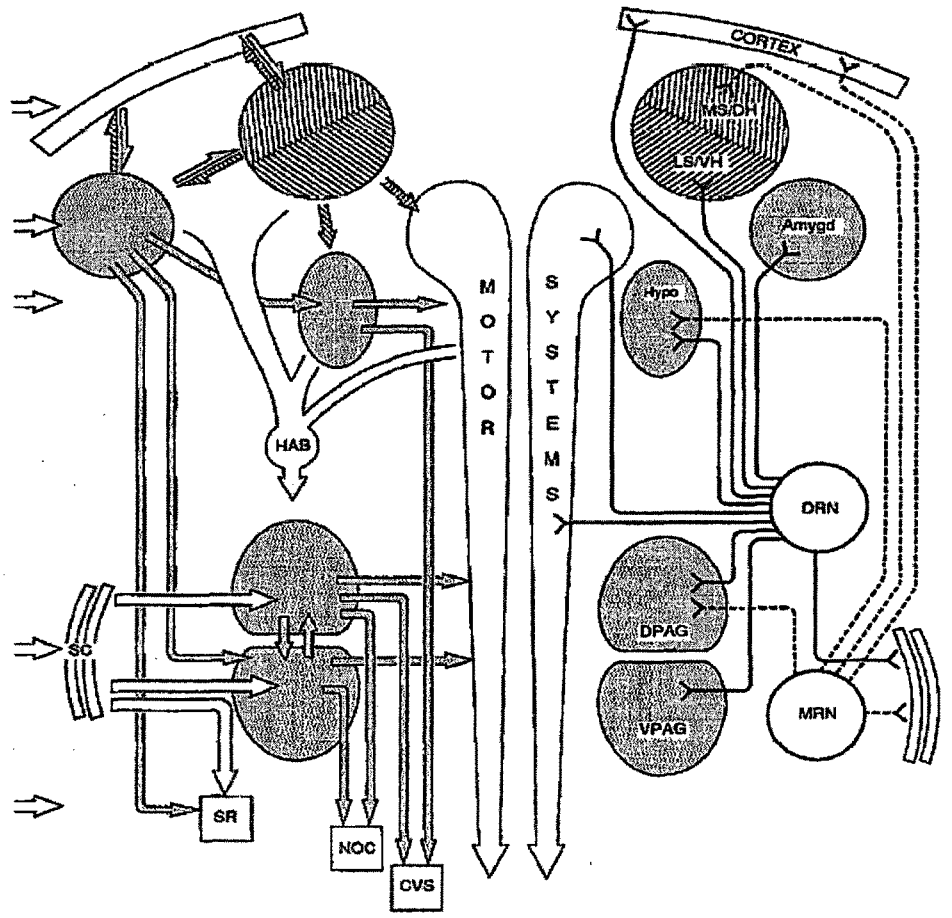


Figure 3: Brain aversive systems (left) and their innervation by Dorsal and Median Raphe nuclei (right). Stippled areas denote structures associated with the 'Defence System' and hatched areas, those associated with the 'Behavioural Inhibition System'. White arrows denote sensory input. Abbreviations: Amygd, amygdala; MD/DH, medial septum/dorsal hippocampus; LS/VH, lateral septum/ventral hippocampus; HAB, habenula; Hypo, hypothalamus; SC, superior colliculus; SR, startle response circuit; NOC, nociceptive control mechanisms; CVS, cardiovascular control mechanisms.

The functions of the ventrol-lateral regions of the PAG (VPAG) are more controversial. VPAG activation is associated with behavioural suppression, immobility and freezing; Fanselow (1991) proposes a role in passive defence, and indeed, the accompanying autonomic changes resemble those seen during passive coping with stress (Bohus *et al.*, 1990), while Lovick (1991, 1993a, 1993b) has suggested a recuperative role following stress or physical exertion. The two roles are not incompatible, given that stimulation in humans caused both pleasant (caudal) and unpleasant (rostra) effects. VPAG stimulation also causes naloxone-sensitive (opioid) analgesia (Fanselow, 1991). The PAG is important for emotional vocalisation, both in the audible and ultrasonic range (Bandler and Depaulis, 1991) and DPAG at least can support secondary conditioning. This occurs when an established aversive CS + is paired with a new neutral stimulus, which, in turn, then becomes a CS+. Fanselow- (1991) commented that such secondary aversive stimuli call forth particularly strong, but poorly directed, emotional reactions and Maier *et al.*, (1993) proposed that this made them excellent candidates for the 'fear of being afraid', which characterises much human pathological anxiety.

The startle response is also viewed as a constituent of the defence system (Davis, 1990). The reflex circuit of acoustic startle has been fully elucidated (Davis, 1990; Davis *et al.*, 1991). Input is via the lateral geniculate nucleus, and the circuit accesses spinal motor systems at the level of the nucleus reticularis pontis caudalis. The experience of startling is highly aversive. A direct pathway from the amygdala gives rise to the potentiation of the startle response by a CS + for shock ('fear'-potentiated startle), while ablation of the deeper layers of the superior colliculus enhances startle and lesion of the substantia nigra prevents 'fear'-potentiated startle by a dopamine-independent mechanism (Davis *et al.*, 1991). The function of startle may be to prime motor output for subsequent rapid movement such as flight. Medullary nuclei are also targets for the defence system. Although until recently little attention was paid to their potential role in aversive behaviour (Lovick, 1993a) they undoubtedly are involved in the somatic signs of anxiety. The production of endogenous opioids after VPAG activation may also play a role in aversive behaviour. Morphine reduced rats' vocal reactions to a present predator while increasing behavioural suppression in an environment associated with a predator (Blanchard *et al.*, 1991). The existence of a 'Behavioural Inhibition System' was proposed by Gray (1982, 1991). He based this system in the septum and hippocampus because lesions to these regions release rewarded behaviour that has been suppressed by the

threat of electroshock 'punishment'. Gray (1982) proposed that this system functioned to suppress behaviour that was under the threat of contingent punishment or of non-reward. The resulting conflict between approach and avoidance drives was seen as fundamental to anxiety, particularly since the 'punished' behaviour is released by benzodiazepines. It is also possible that the Behavioural Inhibition System operates whenever there is a choice to be made with respect to rewarded behaviour.

1.3.4 - 5-Hydroxytryptamine Innervation of Brain Aversive Systems

Figure 3 illustrates the 5-HT innervation of the proposed aversive systems (Geyer *et al.*, 1976; Parent *et al.*, 1981; Beitz *et al.*, 1986; Jacobs and Azmitia, 1992). MRN supplies the dorsal hippocampus and medial septum, the proposed origins of the Behavioural Inhibition System, while DRN innervates the lateral septum and ventral hippocampus, the possible origin of safety signalling. The amygdala, the 'head nucleus' of the Defence System, is almost entirely supplied by DRN, although an input from MRN may innervate the basolateral region. Both nuclei supply the cortex, with a predominant DRN input to frontal cortex and the hypothalamus. The basal ganglia are predominantly supplied by DRN, although again, there is evidence for the existence of some terminals derived from MRN. DPAG and the overlying Superior Colliculus receive input from both DRN and MRN, while only DRN innervates VPAG. Stimulation of DRN by means of the excitatory amino acid DL-homocysteine (DL-H) predominantly inhibited DPAG neurones, an effect that was intensified by local (DPAG) application of paroxetine; this 5-HT uptake inhibitor (Lovick, 1994) inhibited the less frequent excitatory responses. No effect was found after MRN stimulation in this study (Lovick, 1994). Both DPAG and VPAG are also supplied by the medullary raphe nuclei (Beitz *et al.*, 1986). A predominantly inhibitory input to DPAG has been identified coming from NRO, which seems likely to innervate 5-HT_{1A} receptors, together with a lesser, possibly $5HT_2$ receptor mediated, excitation (Lovick, 1993b, 1993c) NRO, and possibly other medullary raphe nuclei, therefore, may be able to modulate DPAG-induced aversion as well as the autonomic components of aversive responses. The afferents to DRN and MRN recently have been reviewed extensively (Jacobs and Azmitia, 1992). DRN and MRN are interconnected and also have connections with other raphe nuclei. The

major limbic input is through the lateral habenula, via an excitatory amino acid pathway that innervates DRN and MRN directly and also terminates on GABAergic interneurones that surround these nuclei. The hypothalamus also has several inputs to DRN and MRN. Cholinergic, adrenergic, noradrenergic (from locus coeruleus), neuropeptide and histaminergic afferents have been demonstrated. The adjacent PAG inputs probably utilise neuropeptides.

1.3.5 - A Theoretical Model

Deakin and Graeff (1991) suggested that different 5-HT pathways and receptor subtypes modulate the neural substrates of depression, panic, and generalized anxiety, respectively (Figure. 4). According to this assumption, the ascending 5-HT pathway that originates in the dorsal raphe nucleus (DRN), runs along the medial forebrain bundle, and innervates the amygdala and frontal cortex facilitates active escape or avoidance behaviors that occur in response to potential or distal threat (Blanchard *et al.*, 1989).

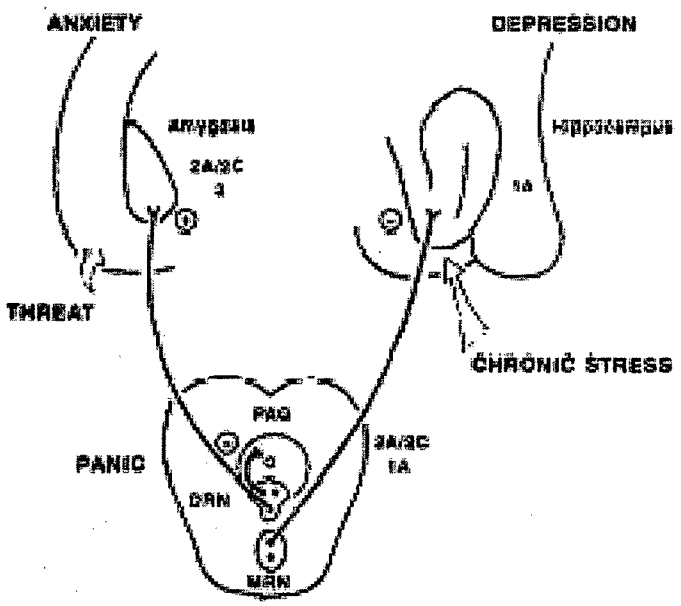


Figure 4: Schematic representation of the role of 5-HT in the modulation of emotions evoked by acute (threat) or chronic stress. 5-HT neurons in the dorsal raphe nucleus (DRN) are supposed to be activated in threatening situations. Their ascending projections facilitate neurons that evaluate danger in the amygdala and inhibit neurons commanding flight in the

dorsal periaqueductal gray (PAG). 5-HT neurons in the median raphe nucleus (MRN) would be activated by persistent and uncontrollable stress. In the hippocampus, 5-HT would promote disconnecting mechanisms that result in more tolerance to stress and, thus, prevent depression.

These behavioral strategies rely on learning and, thus, relate to conditioned or anticipatory anxiety and, possibly, GAD. Postsynaptic 5-$HT_{2A/2C}$ and 5-HT_3, receptors are likely to be activated by this pathway. In turn, the DRN-periventricular pathway innervates the periventricular and periaqueductal gray matter. In these regions 5-HT inhibits inborn fight or flight reactions triggered by proximal danger (Blanchard *et al.*, 1989), acute pain, or asphyxia that may relate to panic disorder. This function of 5-HT is likely to be mediated by both $5HT_{2A/2C}$ and 5-HT_{1A} postsynaptic receptors.

The two pathways discussed above regulate adaptive responses to acute stress. However, there are instances in which aversive stimulation cannot be escaped or avoided, the organism having, thus, to cope with chronic stress. Deakin and Graeff (1991) further suggested that the pathway connecting the median raphe nucleus (MRN) to the hippocampus promotes resistance to chronic stress by disconnecting the aversive events from psychobiological processes underlying appetitive and social behaviors, thus allowing the animal or person to lead an almost normal life despite persistent adversity. Depression supervenes when this coping mechanism fails. 5-HT_{1A} receptors are likely to be the main target of the MRN-hippocampal pathway.

1.3.6 - The dual 5-HT fear hypothesis

A dual role for 5-HT has been suggested by Deakin and Graeff (1991) in the mediation of different types of anxiety. Thus, 5-HT released from nerve terminals from the DRN is supposed to increase learned anxiety at the amygdala, whereas 5-HT released from DRN terminals innervating the DPAG would inhibit unconditioned fear. They argued that a brain system that promotes highly integrated defensive behaviors (in the amygdala) while at the same time inhibiting primitive fight/flight reactions (in the DPAG) would have clear survival value.

1.3.7 - The locus coeruleus and arousal

Autonomic activation and increased arousal are among the earlier psychophysiological responses observed in a state of fear or anxiety. Since the immediate consequences of autonomic activation (e.g, tachycardia) are perhaps the most readily perceived when experiencing a state of fear or anxiety, it has been proposed that the ascending noradrenergic system originating from the locus coeruleus (LC) is the core around which feelings of anxiety are organized (Redmond and Huang, 1979). The LC contains a large proportion of the noradrenaline (NA) cell bodies found in the brain and it is a key brain stem region involved in arousal (Figure 5). It is highly responsive to alerting/stressful stimuli. In rats, cats, and monkeys, increased LC neuronal firing rate is associated with alertness, selective attention to meaningful and/or novel stimuli, and vigilance. The meaning, as well as the intensity of stimuli, seems to be an important factor in LC response. In cats, confrontation with a novel, but non-threatening stimulus, such as a mouse, does not cause a specific increase in LC firing, whereas confrontation with a threatening stimulus (e.g, a dog) causes a marked increase in LC firing. Thus, novelty by itself is not sufficient to activate the LC/NA system, but stimuli that signal reward, as those that signal danger, may activate the system (Southwick *et al.*, 1999). Recent data suggest that a phasic mode of LC activity may promote focused or selective attention, whereas a tonic mode may produce a state of high behavioral flexibility or scanning attentiveness (Aston-Jones *et al.*, 1999). Some LC neurons project to the paraventricular nucleus (PVN) in the hypothalamus and activate the hypothalamo-pituitary-adrenocortical (HPA) axis, triggering or facilitating the stress response associated with increased anxiety (Figure 5). However, although 6-hydroxydopamine lesions of the LC in rats affect the HPA axis response to acute stress, they do not appear to substantially affect its response to chronic stress (Zeigler *et al.*, 1999). Noradrenergic LC neurons also project to the amygdala (mainly to the central nucleus of the amygdala [CeA]), the prefrontal cortex (PFC), the bed nucleus of the stria terminalis (BNST), the hippocampus, the periaqueductal gray (PAG), the hypothalamus, the thalamus, and the nucleus tractus soli-tarius (NTS), which are all areas involved in the fear/anxiety response (Figure 5). The LC is in turn innervated by areas such as the amygdala (which processes fear-related stimuli) and other areas receiving visceral stimuli relayed by the NTS. The LC is therefore in a key position to integrate both

external sensory and internal visceral stimuli and influence stress and fear-related neuroanatomical structures, including cortical areas (Sullivan *et al.*, 1999).

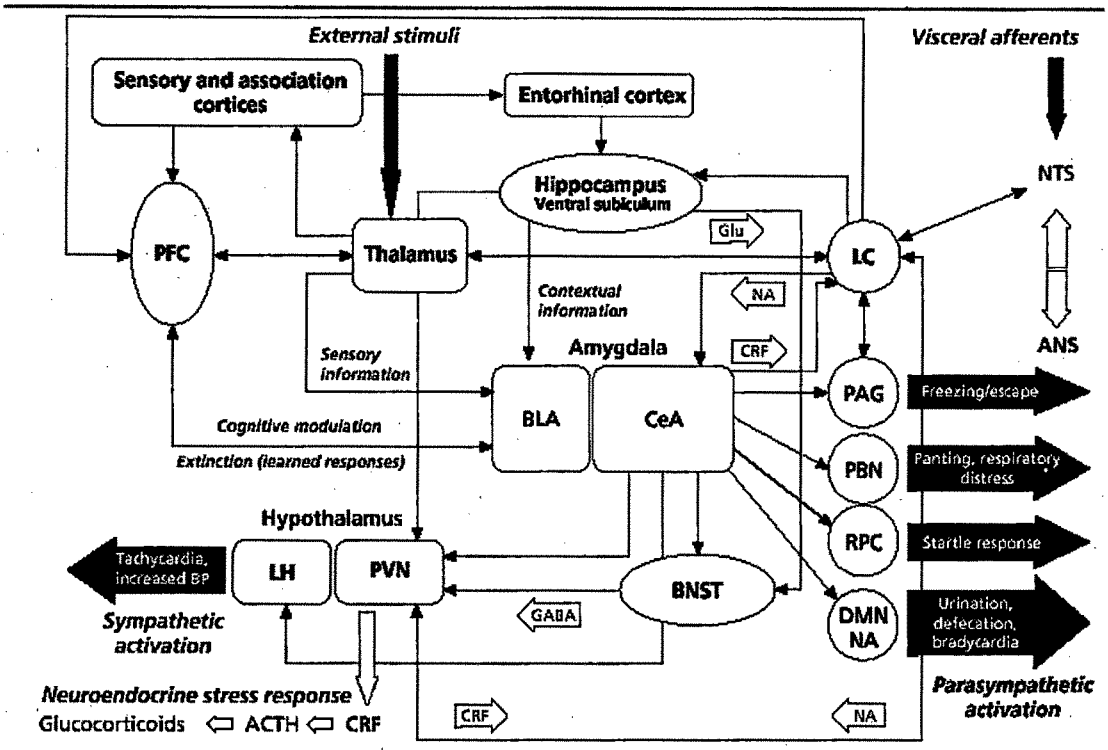


Figure 5: A schematic view of major brain circuits involved in fear and anxiety. External auditory, visual, olfactory, or somatosensory stimuli are relayed by the thalamus to the amygdala and cortex. The basolateral complex (BLA) of the amygdala is the input side of the system, which also receives contextual information from the hippocampal formation (entorhinal cortex, hippocampus, and ventral subiculum). After intra-amygdala processing of the emotional stimuli, the central nucleus of the amygdala (CeA), on the output side, activates the locus ceruleus (LC) and central and peripheral noradrenaline systems (via corticotropin-releasing factor [CRF] neurons), and the hypothalamus (paraventricular nucleus [PVN] and lateral hypothalamus [LH]). The bed nucleus of the stria terminalis (BNST, part of the "extended amygdala") is also a control center for the neuroendocrine system by integrating information originating from both the hippocampus and the amygdala. In addition, the CeA directly activates various midbrain regions or nuclei responsible for different aspects of the fear/anxiety response: freezing or escape (periaqueductal gray

[PAG]), increased respiratory rate (parabrachial nucleus [PBN]), startle (caudal reticulopontine nucleus of the reticular formation [RPC]), and the dorsal motor nucleus of the vagus (DMN) in the medulla, which (together with the lateral hypothalamus) is responsible for the increase in heart rate and blood pressure associated with emotional events. The prefrontal cortex (PFC) processes more elaborate
("Cognitive") information; it modulates the physiological, neuroendocrine, and behavioral responses (via the amygdala), and it is also involved in the extinction of fear- and anxiety-related conditional responses. ACTH, adrenocorticotropic hormone; ANS, autonomous nervous system; BP, blood pressure; GABA,γaminobutyric acid; Glu, glutamate; NA, noradrenaline (neurotransmitter) or nucleus ambiguous (structure); NTS, nucleus tractus solitarius.

1.4 - Neurochemistry of anxiety

Most work in the neurochemistry of anxiety has centered on the monoamines: 5-HT and noradrenaline (NA), the GABA-benzodiazepine (BDZ) receptor complex, and a number of unrelated compounds that are known to provoke anxiety and/or panic on challenges (Table 3). The challenge test work is largely based on human research, with the aim of creating a model for panic disorder. Interest in the GABAergic, serotonergic, and noradrenergic systems originates from the clinical efficacy of compounds affecting them in the treatment of anxiety states and the study of their agonists and antagonists in the provocation of and prevention of anxiety. While the majority of the literature has centered on panic disorder, there is increasing interest in other anxiety states, especially social phobia.

Table 3

Overview of the pharmacological provocation of anxiety

Challenge paradigms	Provocation of panic and anxiety	Mechanisms
Serotonergic	Clorimpramine, selective 5-HT reuptake inhibitors	? Increase in 5-HT
	mCPP	5-HT_2 receptor stimulation
GABAergic	Inverse agonist (FG 7142)	Decrease GABAergic tone
	Antagonists (flumazenil)	Decrease GABAergic tone
Noradrenergic	Yohimbine	Increase NA
	TCAs	Increase NA and 5-HT
	Isoprenaline/NA	Peripheral symptoms
Respiratory	Lactate	? pH changes, ? increase in pCO2
	Bicarbonate	? pH changes, increase in pCO2
	Hypercapnia	Increase in pCO2
	Hyperventilation	Decrease in pCO2
Other	CCK	CCKb receptor in brainstem
	Cognitive	Catastrophic misinterpretation
	CRF	Increase in CRF1 and CRF2

Noradrenergic system

The role of NA, adrenaline, and other sympathomimetics on anxiety has been the subject of interest for many years. The central theme of the noradrenergic theory of anxiety is that increased noradrenergic release leads to anxiety, via what might be called excessive or dysfunctional arousal. Evidence supporting this theory includes: (1) the anxiogenic effects of drugs that increase NA availability, such as amphetamines, cocaine (Louie *et al.*, 1989), and initial treatment with imipramine (Nutt and Glue, 1989); (2) the central role of the noradrenergic locus coeruleus in the control of arousal (Aston-Jones *et al.*, 1994; Smith and Nutt, 1996) and anxiety (Redmond and Huang, 1979); (3) evidence of increased sympathetic activity, correlated with levels of anxiety in exposure paradigms (Ko *et al.*, 1983; Charney *et al.*, 1984); and (4) the anxiogenic and anxiolytic properties of centrally acting selective noradrenergic drugs. The selective β-agonist isoprenaline has been claimed to induce anxiety (Maranon, 1924; Cantrill and Hunt, 1932; Frankenhaeuser *et al.*, 1961) and panic (Rainey *et al.*, 1984; Pyke and Greenberg, 1986), despite the fact that it does not cross the blood-brain barrier. This has led to speculation that peripheral β-adrenoceptors are hypersensitive in panic patients (Rainey *et al.*, 1984). This peripheral effect makes it hard to interpret the true anxiogenic qualities of centrally acting adrenergic drugs, because peripheral symptoms act as a confounding factor. Interest in the role of CNS noradrenergic function has centred on the regulatory role of α-adrenoceptors in the locus coeruleus. Yohimbine is α_2-receptor antagonist that is known to cause anxiety in normal subjects (Goldberg *et al.*, 1983). It is thought to work by antagonising the presynaptic α_2-receptors at noradrenergic synapses, especially those in the locus coeruleus. This breaks the normal feedback loop and leads to increased firing at the locus coeruleus (Charney *et al.*, 1984). The administration of yohimbine to panic patients has been shown to increase anxiety and panic frequency (Charney *et al.*, 1984; Uhde *et al.*, 1983), and interestingly, this is also accompanied by an elevation in the plasma concentration of the NA metabolite 3-methoxy-4-hydroxyphenylglycol, which is higher in the patient group than in the controls, suggesting an increased sensitivity to yohimbine in panic patients. This model has been criticised, however, because yohimbine-induced panics are associated with cortisone release (den Boer and Westenberg, 1993), unlike spontaneous ones. If yohimbine increases anxiety by antagonising

the α_2 presynaptic feedback loop, then one would expect an agonist at this site to reduce locus coeruleus firing and consequently, anxiety. Clonidine is a centrally acting α_2-receptor agonist that has been shown to reduce firing at the locus coeruleus, reduce sympathetic outflow, and decrease anxiety (Reid, 1983).

Serotonergic system

5-HT has been implicated in the neurochemistry of anxiety for a long time, but interest in it has increased dramatically in the past 10 years for a number of reasons. The evidence is confusing and at times contradictory (Handley, 1995; Coplan *et al.*, 1992), but includes the results of challenge studies and the well established clinical effectiveness of serotonergic drugs in the treatment of anxiety (Boyer, 1993; Lydiard *et al.*, 1996). At least some of this confusion is derived from the fact that there are two major serotonergic systems in the brain, the medial raphe nuclei (MRN) and the dorsal raphe nuclei (DRN). These are morphologically distinct, have different afferent and efferent projections (Graeff, 1990; Azmitia and Whitaker, 1995), and function in parallel and together (Tork and Hornung, 1990). Both systems are believed to be important in anxiety, and Grove *et al.* (1997) have proposed a model in which the MRN is important in the modulation of fear and anticipatory anxiety and the DRN in modulating cognitive processes. Work on
5-HT challenge studies in humans have given mixed results. A single dose of a 5-HT_2 agonist (*m*-chlorophenylpiperazine, *m*-CPP) is anxiogenic in patients with panic disorder (Charney *et al.*, 1987), obsessive-compulsive disorder (Zohar *et al.*, 1987), and in normal controls at high enough doses (Charney *et al.*, 1987). Fenfluramine, a 5-HT releasing agent, is also anxiogenic in panic disorder, social phobia, and normal controls at high enough doses (Targum, 1990; Tancer *et al.*, 1994), as is intravenous clomipramine (a serotonergic TCA) (George *et al.*, 1995). However, L-tryptophan and 5-HTP, the precursors of 5-HT, are known to cause sedation and anxiolysis (Charney *et al.*, 1987; Nutt and Cowen, 1987; Westenberg and den Boer, 1989). As with peripheral sympathomimetics, the anxiety response to 5-HT agonists could simply be a cognitive misinterpretation of the side effects produced by these drugs (Kahn *et al.*, 1988a). However, panic patients do have an increased cortisone response to *m*-CPP and fenfluramine (Targum, 1990), but not to clomipramine (George *et al.*, 1995).

The picture is further clouded by the observation that an acute increase in 5-HT leads to increased anxiety, later with anxiolysis on chronic stimulation. It had been proposed (Kahn *et al.*, 1988b) that this was because initial stimulation of hypersensitive 5-HT receptors is followed by down-regulation in response to chronic bombardment. However, animal studies with microdialysis may contradict this idea. Microdialysis measures the overflow of 5-HT into the extracellular space. Most researchers consider this overflow to represent an indirect measure of 5-HT release in the synaptic cleft, although some alternative explanations have been put forward. For all their worth, the microdialysis studies show that 5-HT is not increased in the early stages of treatment with selective 5-HT reuptake inhibitors until there is subsequent down-regulation of presynaptic 5-HT_{1A} autoreceptors (Blier *et al.*, 1990). Buspirone is thought to primarily work on the presynaptic 5-HT_{1A} autoreceptors (Taylor *et al.*, 1985) and, therefore, decrease cell firing and serotonergic drive, although at higher doses, it may also work postsynaptically and, hence, increase panic and anxiety (Frazer and Lapierre, 1987). Buspirone causes activation of locus coeruleus neurons via an α_2 receptor antagonistic action of its metabolite1- (2- Pyrimidinyl)-Piperazine (Engberg, 1989; Cassia, 1986). Collating this information into a coherent theory presents a number of problems, and it is now accepted that the relationship between anxiety and 5-HT is a complex one. Increasingly, the classical view that anxiety was secondary to excessive 5-HT activity is being challenged by more sophisticated models. These place less emphasis on global levels, but different serotonergic neural circuitry and receptors mediating different aspects of anxiety (Deakin and Graeff, 1991). For example, the serotonergic pathway from the DRN to the PAG is particularly involved in unconditioned fear (panic), and that from the DRN to the amygdala and frontal cortex in anticipatory anxiety and conditioned fear (avoidance). The responses of these two systems to changes in availability of 5-HT are quite different. Increases in 5-HT at the PAG are thought to inhibit panic, whereas those at the amygdala are thought to be anxiogenic (Graeff *et al.*, 1996). It is not clear yet whether these different anatomical systems are associated with different 5-HT receptor subtypes that would make pharmacological manipulation possible. Early animal work has suggested that a specific 5-HT_{2C} agonist with anti-panic properties exerts its action through the PAG (Jenck *et al.*, 1996). Some authors have speculated that 5-HT has a central role in the organism's response to adversity and stress, with these different subsystems being adaptations to dealing with

acute and chronic adversity. The failure of these subsystems is thought to be responsible for different clinical syndromes, such as anxiety states and depression (Bell and Nutt, 1998; Deakin and Greaff, 1991).

γ -Aminobutyric acid-benzodiazepine receptor complex

The BDZs and other drugs that work at the GABA receptor have a long history of effective use and abuse in anxiety. Alcohol is perhaps the most widely used anxiolytic, and is active at the GABA-chloride ionophore. Its use to treat normal and pathological anxiety is well recognised, although there is also the question of whether alcohol, in general, and repeated withdrawal, in particular, can cause anxiety disorders such as panic (George *et al.*, 1990). Alcohol, along with barbiturates, BDZs, and other compounds that act at this site (Figure. 6), has an effective and fast anxiolytic action. Since the development of BDZs in the 1960s, interest has focused very much on the high affinity BDZ site, which is situated on and modulates allosterically the GABA-chloride ionophore complex (Braestrup and Squires, 1978; Braestrup *et al.*, 1983). Unlike alcohol and the barbiturates, BDZs have no direct action on the chloride ionophore itself, but work by augmenting the effect of the endogenous excitatory aminoacid GABA. This phenomenon explains the relative safety of the BDZs, as they are unable to do more than maximize physiological stimulation. One of the reasons why this receptor, and its role in anxiety, generates a great interest in researchers is that it is unique in showing bi-directional agonism.

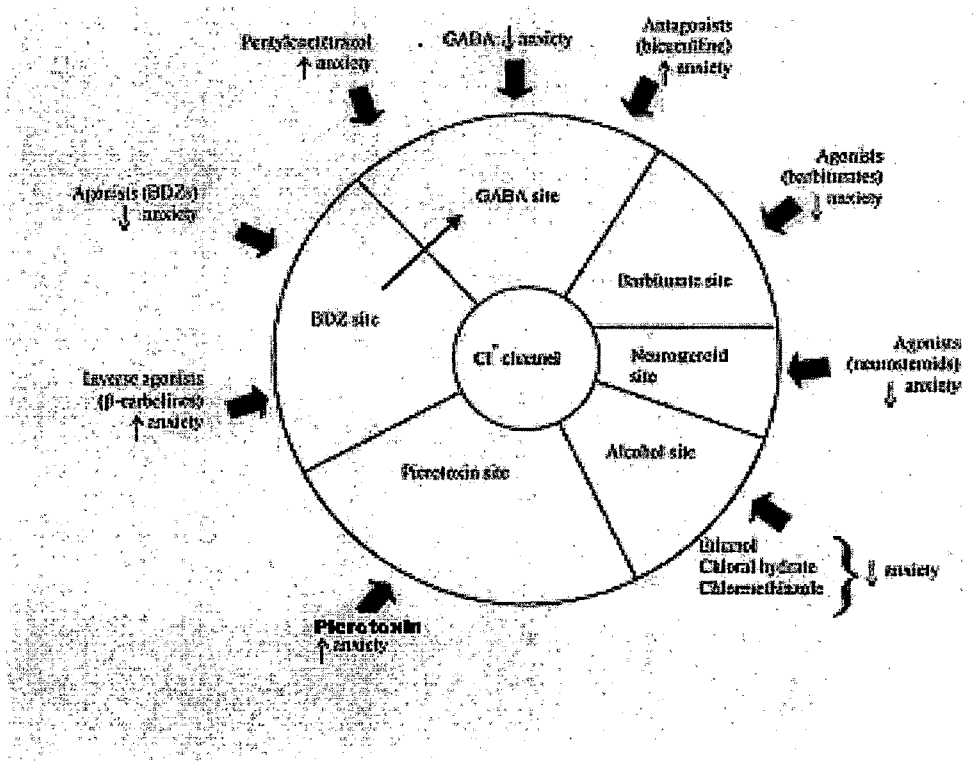


Figure 6: The role of GABA-A receptor in anxiety. Actions that mimic or potentiate those of GABA result in increase of chloride flux and reduction in anxiety. Opposite actions that reduce GABA function increase anxiety. Reproduced from Argyropoulos and Nutt (1999)

As well as agonists at its site (i.e., the conventional BDZs), there are also antagonists and inverse agonists (Jackson and Nutt, 1992). Antagonists such as flumazenil have little action of their own, but block the actions of both agonists and inverse agonists. Inverse agonists, such as the β-carboline FG 7142 and BDZ Ro15-3505, are profoundly anxiogenic in humans (Dorow *et al.*, 1983; Gentil *et al.*, 1990). This unique quality has raised interest in the idea that endogenous agonists or inverse agonists may have a role in the genesis of anxiety, or that abnormality in the BDZ receptor may underlie some anxiety disorders. Candidate inverse agonists included tribulin (Clow *et al.*, 1983) and diazepam-binding inhibitor (Barbaccia *et al.*, 1986). Tribulin was identified in human urine and found in increased concentrations in patients after lactate induced panic (Clow *et al.*, 1988a, 1988b), but studies of cerebrospinal

fluid diazepam-binding inhibitor showed increased concentration in depressives (Barbaccia *et al.*, 1986), but not panic patients (George *et al.*, 1990). Interest in the possible role of these compounds waned when it was shown that panic patients find a challenge with flumazenil, an antagonist, markedly anxiogenic when compared with healthy controls (Nutt *et al.*, 1990). These findings refute a significant role for endogenous inverse agonists, and point towards either an endogenous agonist or receptor abnormality. Recently, endogenous agonists, or endozapines, have been isolated from the rat and human brain (Rothstein *et al.*, 1992a, 1992b). While similar compounds are postulated to have a role in idiopathic recurrent stupor (Tinuper *et al.*, 1992), their role in anxiety disorders is yet to be delineated. The most exciting of the endogenous agonists identified so far are the neurosteroids. They act as allosteric modulators of the GABA receptor complex, but they also affect the receptors of the excitatory amino acid N-methyl-D-aspartate (Baulieu, 1998). Fluctuations of gonadal steroids almost certainly regulate anxiety (Wilson, 1996). This is especially true during periods when these fluctuations are more pronounced, such as the menstrual cycle, pregnancy, and the postpartum. There is also animal evidence that progesterone, a naturally occurring gonadal steroid, is metabolized to a neurosteroid compound that potentiates the function of GABA. On the contrary, other gonadal steroids such as estrogens do not appear to modify GABA function (Wilson, 1996).

1.4.1 - Pharmacological provocation of anxiety

Psychological, physiological, and pharmacological methods can be used to provoke panic attacks in individuals, with varying selectivity for patients with panic and other anxiety disorders. Three agents cholecystokinin (CCK), lactate, and hypercapnia have been of focus of research interest.

Cholecystokinin (CCK)

CCK is found in relatively high concentrations in the mammalian brain, where it is believed to act as a neurotransmitter and/or neuromodulator (Rehfeld, 1985). The brain contains two pharmacologically distinct receptors for CCK. The CCKa receptor is the least numerous in the brain, being located mainly in the gallbladder and pancreas. It shows high affinity for the octopeptide fragment, CCK-8.The CCKb subtype is primarily located in the brain, and has high affinity for the tetrapeptide CCK-4 and pentagastrin, a pentapeptide synthetic analogue, as well as CCK-8. Interest in the anxiogenic qualities of CCK occurred when first rats (Csonka *et al.*, 1988) and later healthy human volunteers were observed to have panic attacks when given CCK-4 intravenously.

Sodium lactate

The observation that anxious individuals produce more lactate on exercise than do healthy controls (Jones and Mellersh, 1946) led to later work showing that lactate infusions could cause severe anxiety in susceptible individuals (Pitts and McClure, 1967). The mode of action of lactate-induced panic has been the subject of much interest. Early theories postulated that the anxiogenic qualities of lactate were secondary to hypocalcaemia (Pitts & McClure, 1967). This was later discounted by the discovery, firstly, that the degree of hypocalcaemia is slight (Grosz and Farmer, 1969), and, secondly, that infusions of calcium chelating agents produced tetany, but not anxiety (Pitts and Allen, 1979). Carr and Sheehan (1986) proposed that the secondary alkalosis led to cerebral vasoconstriction, which, in turn, led to cerebral ischaemia. This, coupled with the passive diffusion of lactate, causes a rise in the lactate to pyruvate ratio and subsequent fall in pH in both the brain and areas outside the blood-brain barrier, such as the chemoreceptor zones in the medullary areas. The crux of their argument is that panic patients have a supersensitivity in their medullary chemoreceptors to changes in pH, and hence, are sensitive to minor changes. The theory predicts that in normal controls, panic would occur if the medullary pH changed enough. The problems with this theory are that (1) it is not clear that extracellular changes in pH are

mirrored intracellularly, and may not occur as predicted (Ritter *et al.*, 1990) and (2) clinical evidence suggests that hypercapnia is a necessary prerequisite for the experience of anxiety. A second theory was that it was the metabolic alkalosis and secondary hypercapnia that were aetiological in the panic (Liebowitz *et al.*, 1984; Gorman *et al.*, 1988). Infused lactate is metabolised to bicarbonate, leading to a metabolic alkalosis. One would expect that this would lead to compensatory hypoventilation, not hyperventilation. However, the bicarbonate is further metabolised to CO_2, which then permeates the blood-brain barrier, stimulates the ventral medullary chemoreceptors, and causes hyperventilation. Interestingly, increasing brain CO_2 concentration causes strong activation of the locus coeruleus (Elam *et al.*, 1981), which via central noradrenergic activation could lead to the cognitive features of panic. This theory has foundered on the observation that infusion of D-lactate, the optical isomer of L-lactate, which is not metabolised to CO_2, is also panicogenic (Gorman *et al.*, 1990). Lactate metabolism, therefore, is not necessary for induction of panic or hyperventilation.This led to a search for other explanations of the panicogenic action of lactate. These include the possible augmentation of 5-HT uptake by lactate (Lingjaerde, 1985)

Hypercapnia

Hypercapnia can induce panic. This can be done by rebreathing air, by breathing 5-7% CO_2 in air (Gorman *et al.*, 1988), or even by a single deep breath of 35% CO_2 (Van Den Hout and Griez, 1984; Griez *et al.*, 1987). The quality of sensation in hypercapnia is said to be similar to that in spontaneous panics. If severe enough, hypercapnia will induce panic in anybody, and sensitivity to CO_2 varies with personality (Waeber *et al.*, 1982) and with cognitive interpretation (Sanderson *et al.*, 1989). One theory (Carr and Sheehan, 1984) is that a fall in pH at the brainstem is responsible. In hypercapnia, this is produced directly by the respiratory acidosis, while in hyperventilation; the respiratory alkalosis leads to a secondary cerebral vasoconstriction, which causes a localised decrease in pH at the brainstem.

Corticotropin releasing factor (CRF)

Corticitropin releasing factor is a 41 amino acid peptide plays an essential role in coordinating endocrine, autonomic, immune and behavioral responses to stress (Brown and Fischer, 1990; De Souza, 1995; Koob *et al.*, 1993; Takahashi and Kalin, 1989; Vale *et al.*, 1981). CRF and urocortin, a CRF like peptide, are widely distributed throughout the mammalian brain (Cummings *et al.*, 1983; Kozeiz *et al*, 1998; Sawchenko and Swanson, 1990; Swanson *et al.*, 1983; Vaughan *et al.*, 1995). These peptides exert their biological actions via two major G-protein coupled seven transmembrane domain receptor subtypes known as CRF_1 (Chang *et al.*, 1993; Chen *et al.*, 1993; Vita *et al.*, 1993) and CRF_2 (Lovenberg *et al.*, 1995; Perrin *et al.*, 1995). The CRF_2 receptor has α and β splice variants. In addition a $CRF_{2\gamma}$ receptor is found in human brain (Kostich *et al.*, 1998). Studies demonstrate that CRF_1 and CRF_2 receptors have distinct pharmacological profiles and unique distribution patterns in brain and peripheral tissues (Dautzenberg *et al.*, 2001; Dieterich *et al.*, 1997). In rats, high densities of CRF_1 receptors are found in the pituitary, brain stem, cerebellum, amygdale, and cortex whereas $CRF_{2\alpha}$ receptors are found predominantly in the lateral septum, venteromedial hypothalamus, and olfactory bulb (Chalmers *et al.*, 1995; Primus *et al.*, 1997).

1.5 - Hypertension

1.5.1 - Mineralocorticoid induced hypertension (Endocrine hypertension)

Mineralocorticoids cause retention of Na^+ and water in the body until escape diuresis occurs due to increased pressure on the kidneys. No further retention of sodium and water occurs, but general level of body sodium and water is slightly raised. Selye *et al* was the first to demonstrate that deoxycorticosterone acetate (DOCA) produces hypertension in rats (Selye *et al.*, 1957). There is increased DOCA-induced reabsorption of salt and water leading to increased blood volume and hence increased blood pressure. There is also increased secretion of vasopressin leading to water retention and vasoconstriction. In addition, altered activity of RAAS leads to increased sympathetic activity (Dahl *et al.*, 1960; Hakim and Goyal, 2000). Rats, especially female and young, are prone to DOCA-salt induced hypertension (Dahl *et al.*, 1960; Rathod *et al.*, 1997). This type of hypertension can also be produced in dogs and

pigs. Other mineralocorticoids (e.g., aldosterone) and glucocorticoids can also produce this type of hypertension (Seyle *et al.*, 1957). DOCA induced hypertension is salt dependent since neither administration of DOCA nor partial removal of renal mass is effective in increasing blood pressure when applied without salt administration (Dahl *et al.*, 1960). To produce hypertension, rats weighing about 100 g are kept on a diet high in sodium chloride and drinking water is replaced by 2% sodium chloride solution ad libitum. After they attain a weight of about 250 gm, they are given DOCA dissolved in sesame seed oil at a dose of 10 mg/kg, twice weekly for 43 days (Rathod *et al.*, 1997; Santhoshkumari *et al.*, 1991).
In another method, unilateral nephrectomy is performed followed by DOCA administration (Katholi *et al.*, 1980) can be produced by the following methods:
All operated rats receive an injection of ampicillin (10 mg/kg, i.m.) daily for 5 days and local application of neosporin-H to prevent infection. A week later, DOCA (25 mg/kg/week, s.c.; for 5 weeks) dissolved in cotton seed oil, is injected into nephrectomised rats. Alternatively, nephrectomised rats could receive DOCA from silicon rubber implants (200 mg/rat) implanted subcutaneously. NaCl (1.0 %) solution is substituted for drinking water and given ad libitum (Rathod *et al.*, 1997).

1.5.2 - Dietary hypertension

Increased salt intake

Physiologically, normal kidney has the ability to excrete easily the daily salt load without allowing a marked rise in extracellular volume. However, general epidemiological data have shown that higher the average sodium intake in a given population, the greater will be the prevalence of hypertension. Chronic ingestion of excess salt produces hypertension in rats, which mimics human hypertension morphologically (Dahl *et al.*, 1960). High salt intake hypertension has been produced in rats, rabbits and chicks by replacing drinking water with 1-2% sodium chloride for 9-12 months (Boura and Green, 1964; Rathod *et al.*, 1997). High salt intake along with unilateral nephrectomy produces accelerated hypertension within 3-4 weeks in dogs (Coleman *et al.*, 1975). Accelerated renal hypertension in rats is produced by applying a 'figure of eight' ligature to one kidney and removing the other in a single stage

operation performed under strict aseptic conditions using pentobarbitone sodium (20 mg/kg, i.p.) and ether anesthesia. The drinking water is replaced with physiological saline for three weeks and the animals are used 4 to 5 weeks after the operation (Sharma, 1985).

1.5.3 - Fructose Induced Hypertension

Fructose is widely present in numerous foods. It has been commonly used as a sweetener and promoted as being useful for weight reduction, exercise endurance and diabetes (Dai and McNeill, 1995). It has been demonstrated that hypertension develops when normal rats are fed a fructose-enriched diet (Verma *et al.*, 1994; Dai and McNeill, 1995; Suzuki *et al.*, 1997). Hypertension develops when normal rats are fed a fructose-enriched diet as early as 2 weeks after initiation of the diet.

Fructose and hypertension

Similar to insulin resistance and hyperlipidemia, many published experiments have shown that high-fructose diets induce hypertension in animals, including rodents (Erlich and Rosenthal, 1995; Suzuki *et al.*, 1997; Verma *et al.*, 1994) and dogs (Martinez *et al.*, 1994). In fact, fructose-fed rats are frequently used as a model for studying the effects of pharmacologic agents for treating hypertension (>50 studies during the past 5 years). The mechanism of fructose-induced hypertension is not well understood, but such factors as uric acid production (Reiser, 1985), hyperinsulinemia (Daly *et al.*, 1997), aldehyde formation (Vasdev *et al.*, 1998), and altered vascular reactivity (Verma *et al.*, 1996) has been implicated. Takagawa *et al* (Takagawa *et al.*, 2001) showed that long-term (40 weeks) fructose feeding impaired vascular relaxation in the mesenteric arteries of male Sprague-Dawley rats. Fructose feeding induced hypertension in normal-fed and high salt–fed rats and was associated with an increased expression of the angiotensin II type 1 receptor in adipose tissue (Giacchetti *et al.*, 2000).

Compared with individuals with normal blood pressure, persons with high blood pressure are relatively glucose intolerant (Reaven, 1988). Additionally, lowering blood pressure in hypertensive individuals does not necessarily reduce the degree of glucose intolerance and

hyperinsulinemia. Two potential explanations for how insulin resistance and hyperinsulinemia could lead to an increase in blood pressure are as follows:

1) Increases in sympathetic neural outflow and plasma catecholamine concentrations associated with increased plasma insulin concentrations, and

2) Insulin action at the level of the proximal tubule to increase fluid reabsorption (Reaven and Hoffman, 1990).

Because hypertension is a well-known comorbidity associated with obesity, insulin resistance, hyperinsulinemia, and hyperlipidemia, it is important to determine the effects of fructose consumption on blood pressure in human subjects.

The intake of dietary fructose has increased markedly as a result of the steady increase in added sugars in the American diet (Coulston and Johnson, 2002). In the past, fructose was considered to be beneficial in the dietary management of diabetes mellitus and insulin resistance because fructose ingestion results in smaller postprandial glycemic and insulin excursions than do glucose and complex carbohydrates (Glinsmann and Bowman, 1993). In light of the information presented here, a cautionary note is warranted.

Fructose has been implicated as a contributor to nearly all of the classic manifestations of the insulin resistance syndrome. Insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertension, and hyperlipidemia are associated with fructose intake in animal models. The data in humans are less clear, perhaps in part because the effects of fructose are often compared with those of sucrose, which is composed of 50% fructose. Other complicating factors obscuring the effect of dietary fructose on metabolic indexes include the duration of the studies, the age and the sex of the subjects tested, and the state in which the measurements are made (i.e. fasting or postprandial).

A considerable amount of research needs to be done to more completely appreciate the effect of fructose in the American diet. In the meantime, a prudent approach concerning recommendations for dietary fructose would consider the following two points. First, added fructose (in the forms of sucrose and HFCS) does not appear to be the optimal choice as a source of carbohydrate in the diet. Small amounts of added fructose are probably benign and may even have some favorable metabolic effects. However, on the basis of the available data regarding the endocrine and metabolic effects of consuming large quantities of fructose and the potential to exacerbate components of the insulin resistance syndrome, it is preferable to

primarily consume dietary carbohydrates in the form of glucose (free glucose and starch). This may be particularly important in subjects with existing hyperlipidemia or insulin resistance who could be more susceptible to the adverse metabolic effects of fructose. Second, the concerns rose about the addition of fructose to the diet as sucrose or HFCS should not be extended to naturally occurring fructose from fruit and vegetables. The consumption of fruit and vegetables should continue to be encouraged because of the resulting increased intake of fiber, micronutrients, and antioxidants. In addition, the intake of naturally occurring fructose is low, ≈15 g/d, and is unlikely to contribute significantly to the untoward metabolic consequences associated with the consumption of large amounts of fructose.

1.5.4 - Adrenal gland and hypertension

The adrenal gland is involved in the production of a variety of steroid hormones and catecholamines that influence blood pressure. Thus, it is not surprising that several adrenal disorders may result in hypertension. Many of these disorders are potentially curable or responsive to specific therapies. Therefore, identifying adrenal disorders is an important consideration when elevated blood pressure occurs suddenly or in a young person, is severe or difficult to treat, or is associated with manifestations suggestive of a secondary form of hypertension. Because these occurrences are relatively rare, it is necessary to have a high index of suspicion and understand the pathophysiology on which the diagnosis and treatment of these problems is based. Three general forms of hypertension result from excessive production of mineralocorticoids, glucocorticoids, or catecholamines.

PHYSIOLOGIC MECHANISMS IN ADRENAL HYPERTENSION

Disorder	Cause	Pathophysiology	Pressure mechanism
Primary aldosteronism	Autonomous hypersecretion of aldosterone (hyperminer-alocorticoidism)	Increased renal sodium and water reabsorption, increased urinary excretion of potassium and hydrogen ions	Extracellular fluid volume expansion, hypokalemia (?), alkalosis
Cushing's syndrome	Hypersecretion of cortisol (hyperglucocorticoidism)	Increased activation of mineralocorticoid receptor (?), increased angiotensinogen (renin substrate) concentration	Extracellular fluid volume expansion (?), increased angiotensin II (vasoconstriction and increased peripheral resistance)
Pheochromocytoma	Hypersecretion of catecholamines	Vasoconstriction, increased heart rate	Increased peripheral resistance, increased cardiac output

Table 4: The causes and pathophysiologies of the three major forms of adrenal hypertension and the proposed mechanisms by which blood pressure elevation results.

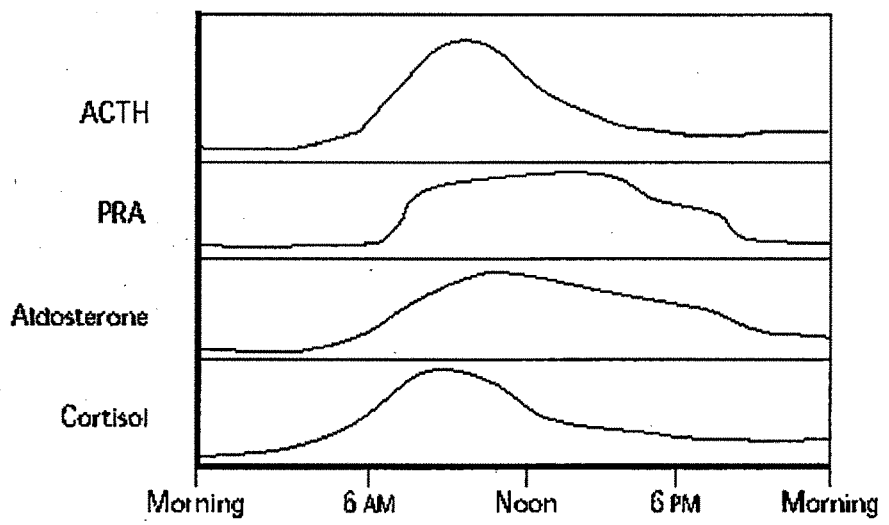


Figure 7: Circadian rhythmicity of steroid production and major stimulatory factors. Aldosterone and cortisol and their respective major stimulatory factors, plasma renin activity (PRA) and adrenocorticotropic hormone (ACTH), demonstrate circadian rhythms. The

lowest values for all of these components are normally seen during the sleep period when the need for active steroid production is minimal. ACTH levels increase early before awakening, stimulating cortisol production in preparation for the physiologic changes associated with arousal. PRA increases abruptly with the assumption of the upright posture, followed by an increase in aldosterone production and release. Both steroids demonstrate their highest values through the morning and early after-noon. Cortisol levels parallel those of ACTH, with a marked decline in the afternoon and evening hours. Aldosterone demonstrates a broader peak, reflecting the postural stimulus of PRA.

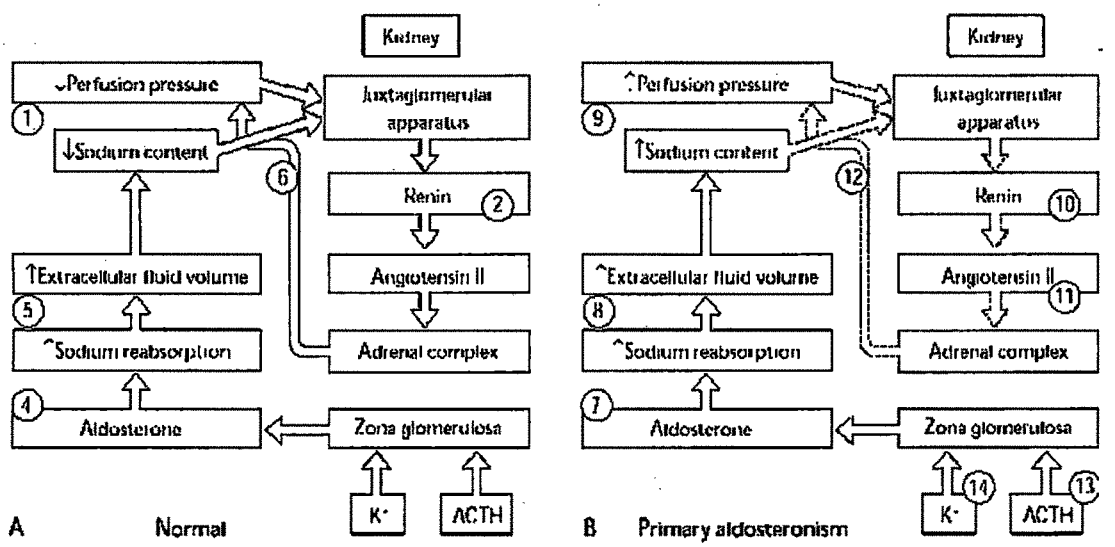


Figure 8: Control of mineralocorticoid production. **A,** Control of aldosterone production under normal circumstances. A decrease in renal perfusion pressure or tubular sodium content (1) at the level of the juxtaglomerular apparatus and macula densa of the kidney triggers renin release (2). Renin acts on its substrate angiotensinogen to generate angiotensin I, which is converted rapidly by angiotensin-converting enzyme to angiotensin II. Angiotensin II then induces peripheral vasoconstriction to increase perfusion pressure (6) and acts on the zona glomerulosa of the adrenal cortex (3) to stimulate production and release of aldosterone (4). Potassium and adrenocorticotropic hormone (ACTH) also play a minor role

in aldosterone production in some circumstances. Aldosterone then acts on the cells of the collecting duct of the kidney to promote reabsorption of sodium (and passively, water) in exchange for potassium and hydrogen ions excreted in the urine. This increased secretion promotes expansion of extracellular fluid volume and an increase in renal tubular sodium content (5) that further suppresses renin release, thus closing the feedback loop (servomechanism). **B,** Abnormalities present in primary aldosteronism. Autonomous hypersecretion of aldosterone (7) leads to increased extracellular fluid volume expansion and increased renal tubular sodium content. These elevated levels are a result of increased renal sodium and water reabsorption (8) at the expense of increased potassium and hydrogen ion excretion in the urine. The increase in sodium and volume then increase systemic blood pressure and renal perfusion pressure and sodium content (9), thereby suppressing further renin release (10) and angiotensin II production (11). Thus, in contrast to the nor-mal situation depicted in *panel* A, the levels of angiotensin II are highly suppressed and therefore do not contribute to an increase in systemic blood pressure (12). In primary aldosteronism, ACTH (13) has a dominant modulatory role in influencing aldosterone production and hypokalemia, resulting from increased urinary potassium exchange for sodium, which has a negative effect on aldosterone production (14).

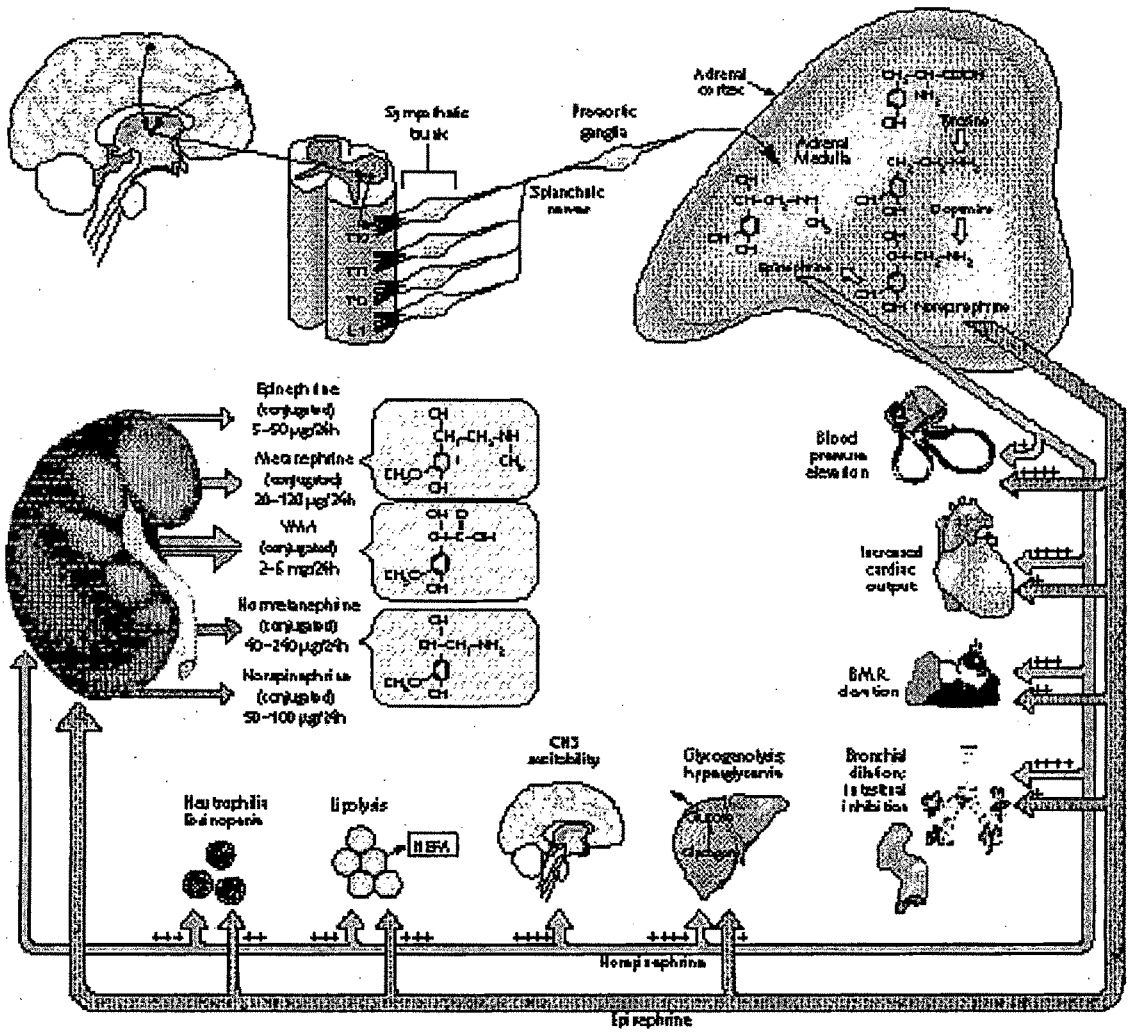


Figure 9: Synthesis, actions, and metabolism of catecholamines. Depicted is the synthesis of catecholamines in the adrenal medulla [9]. Epinephrine is only produced in the adrenal and the organ of Zuckerkandl at the aortic bifurcation. Norepinephrine and dopamine can be produced and released at all other parts of the sympathetic nervous system. The kidney is the primary site of excretion of catecholamines and their metabolites, as noted here. The kidney also can contribute catecholamines to the urine. The relative contributions of norepinephrine and epinephrine to biologic events are noted by the *plus signs*. BMR—basal metabolic rate; CNS—central nervous system; NEFA—nonesterified fatty acids; VMA—vanillylmandelic acid.

[Taken from Adrenal causes of hypertension- Myron S Weinberger]